

Figure 1

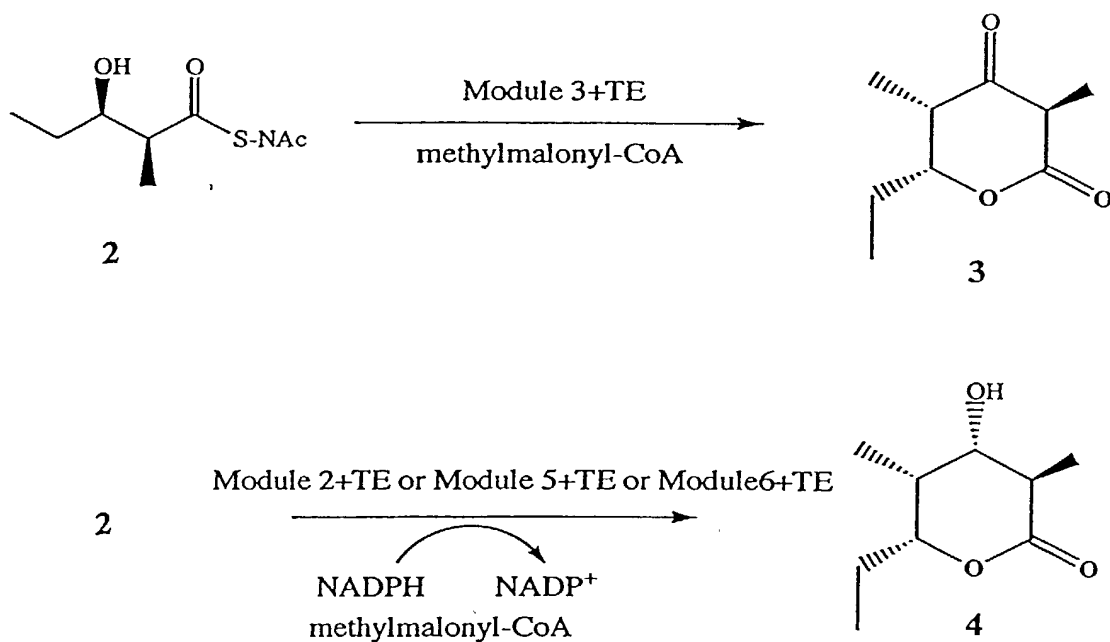
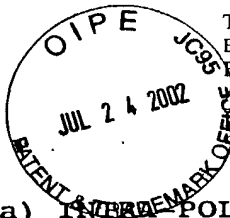


Figure 2



Title: METHODS TO MEDIATE POLYKETIDE SYNTHASE MODULE
EFFECTIVENESS
First Inventor: Rajesh GOKHALE
Application No.: 10/091,244
Pocket No.: 30062-2004620
Sheet 3 of 22

10/091,244 10/091,244 10/091,244

(a) INTER-POLYPEPTIDE LINKER

M2ery: GGATGAEEQAAPATT..APVD
M4ery: VGDAD..QAA.VRVVGAA.DES
M6ery: VGAAEAEEQA.PALVREVPKDDAD
M2rif: FGSA.A.NR.PAEIGTAAAE
M3rif: LG..ER.PAAPAPVTRDVS
M5rif: GETVAGAPATPVTTVADAG
M3rap: .ELFTGENPAPVRGPVSAVGQD
M4rap: .ELFTGENPAPVRGPVSVVGQD
M7rap: .ELFTGENPAPVRGPVSA.GQD

(b) N-TERMINAL INTER-POLYPEPTIDE LINKER

M3ery:VTD SE KVAEYLRR .ATLIDLRAAR QRIRE..LES
M5ery: MSGDNGM.TE E.KLRRYLKR TVT.ELDSVT ARLRE..VEH RAG
M4rif:MSAPNE QIVDAL.R ASLKE....N VRLQQENSAL AAAAA
M7rif:VSASYE KVVEAL.R KSLEE....V GTLKKRNRQL ADAAG
M8rif:V.AD EGQLRDYLKR .AIADARDAR TRLRE..VEE QAR
M9rif:MATD E.KLLKYLR .VTAEHLS.. ..LRKQGARH .AD
M5rap:MR.. EDQLLDAL.R KSVKE....N ARLRKANTSL RAAMD
M11rap:M.PEQD KVVEYL.R WATAELHTTR AKL.....EA LAAANT

Figure 3

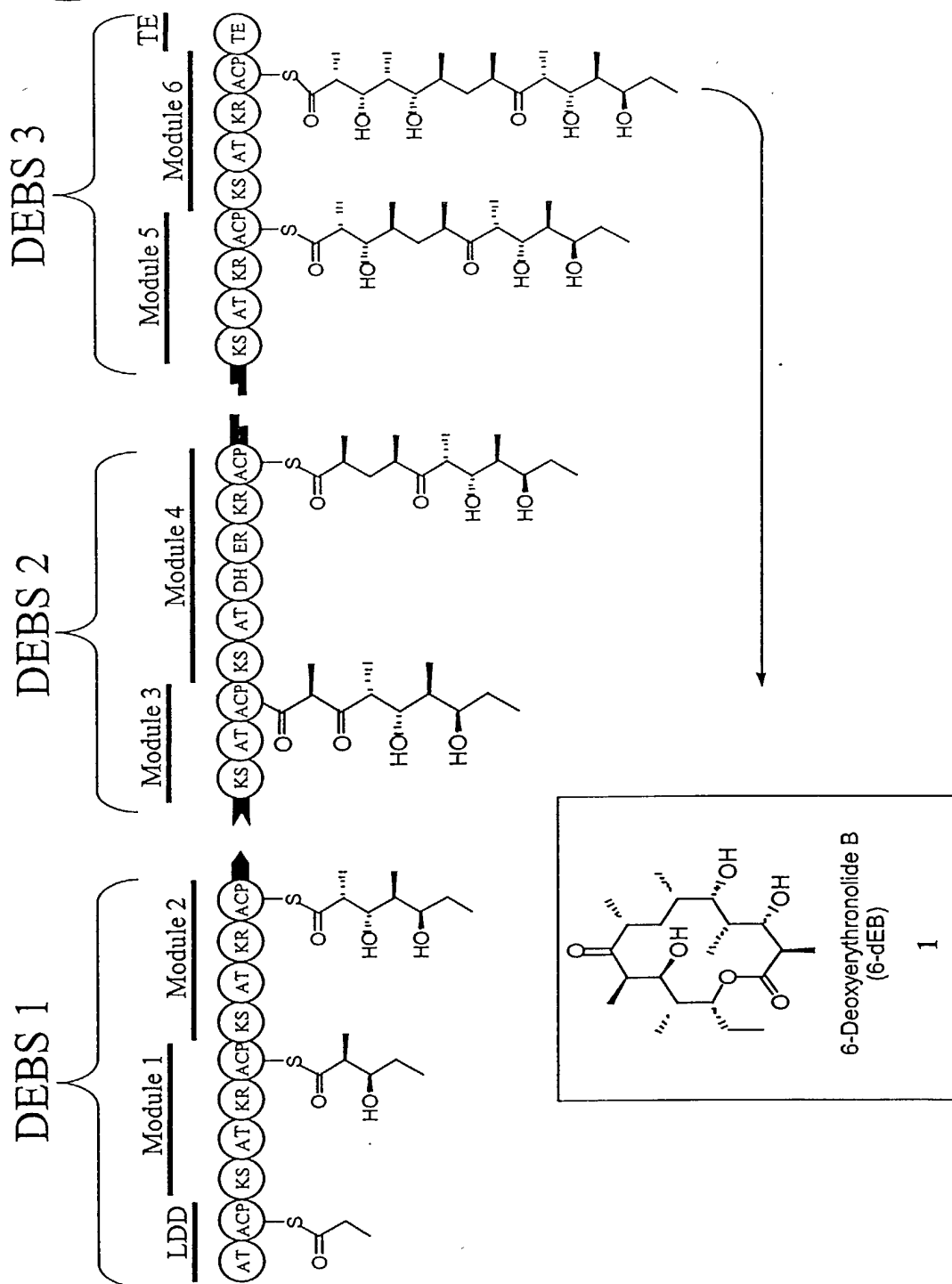


Figure 4



Title: METHODS TO MEDIATE POLYKETIDE SYNTHASE MODULE
EFFECTIVENESS
First Inventor: Rajesh GOKHALE
Application No.: 10/091,244
Docket No.: 30062-2004620
Sheet 5 of 22

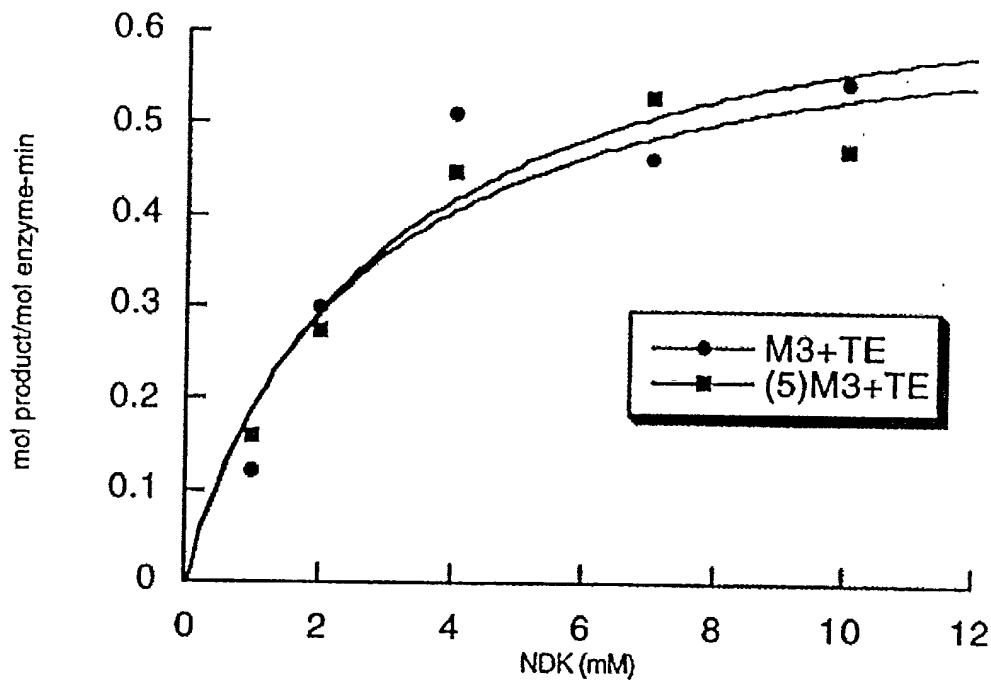
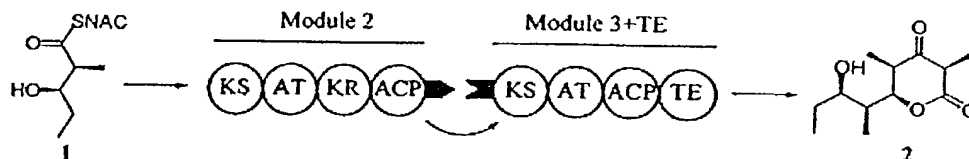


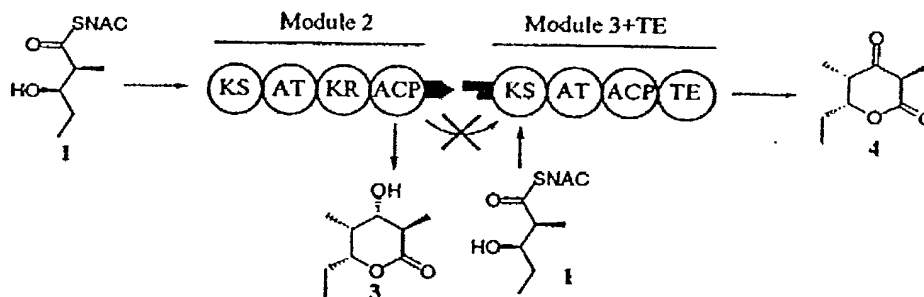
Figure 5



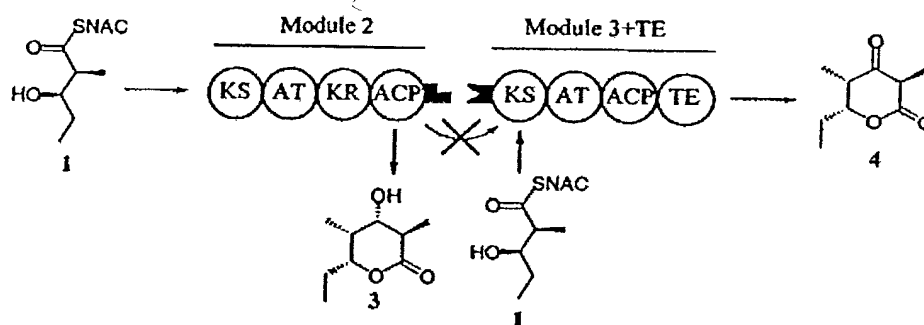
A. M2 and M3+TE



B. M2 and (5)M3+TE



C. M2(4) and M3+TE



D. M2(4) and (5)M3+TE

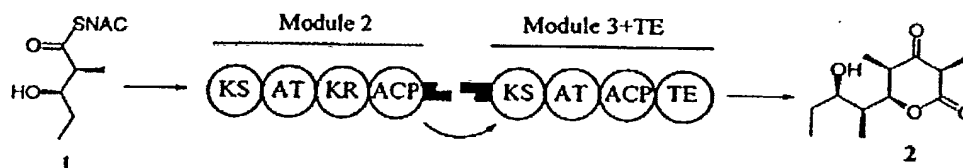


Figure 6

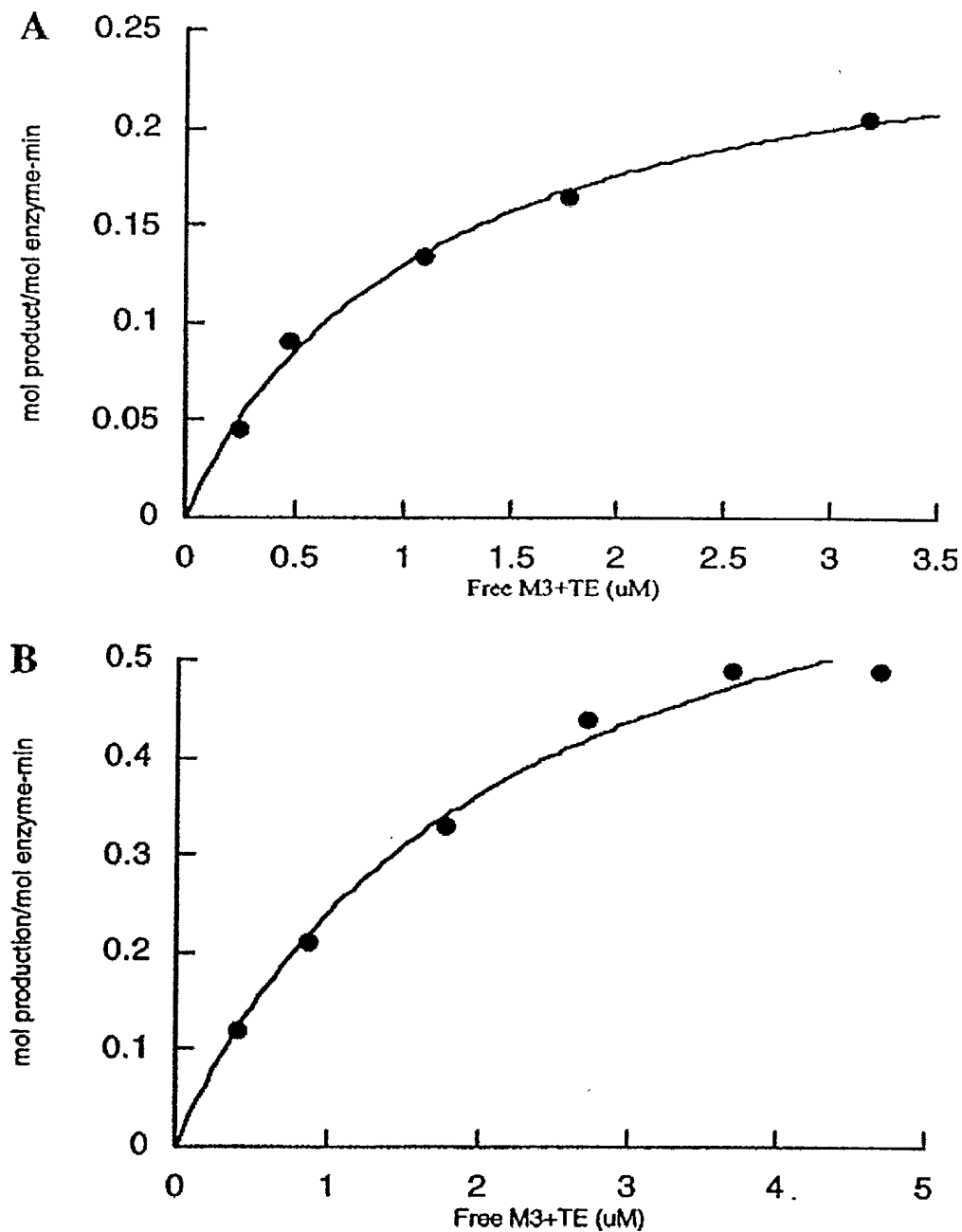
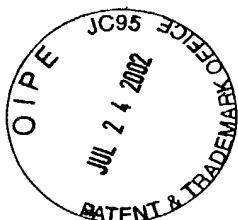


Figure 7

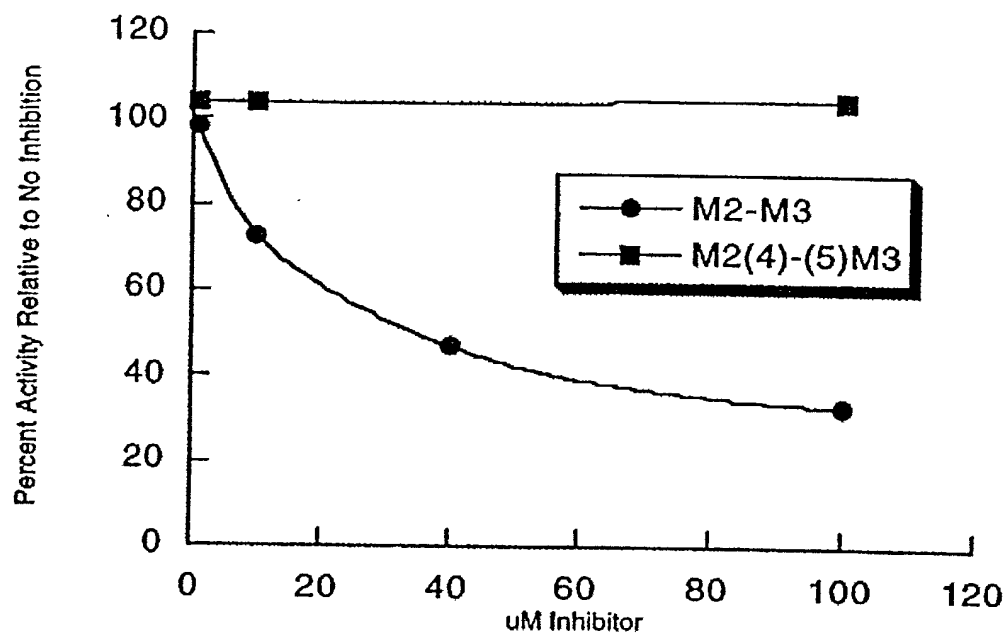


Figure 8

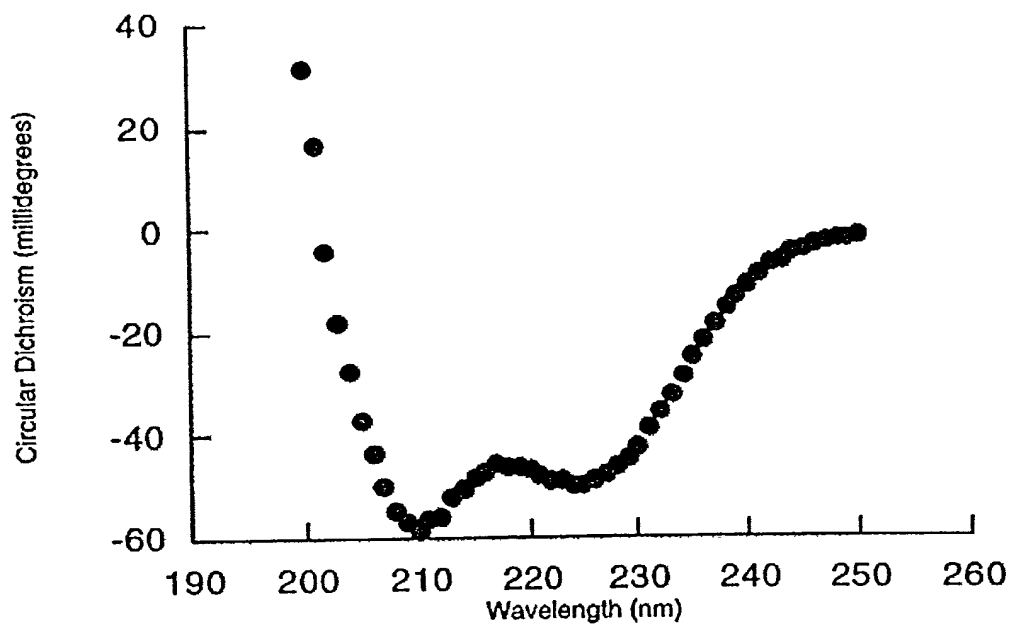
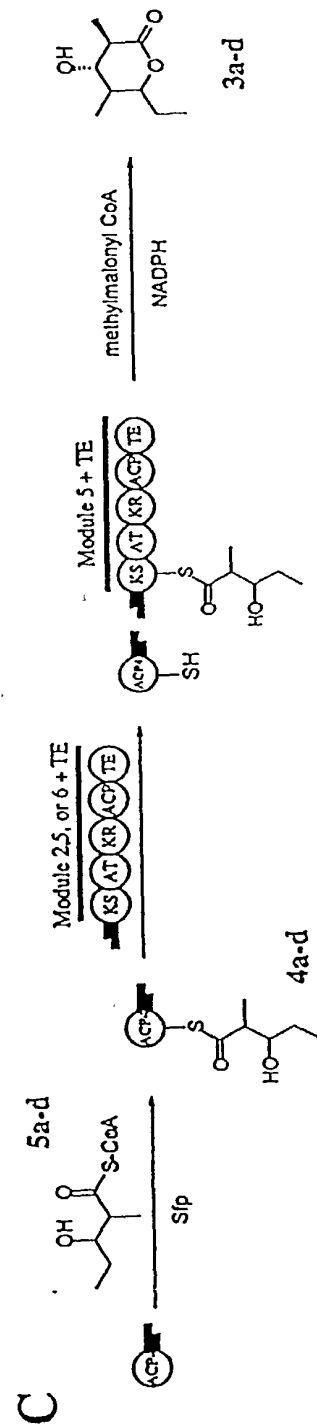
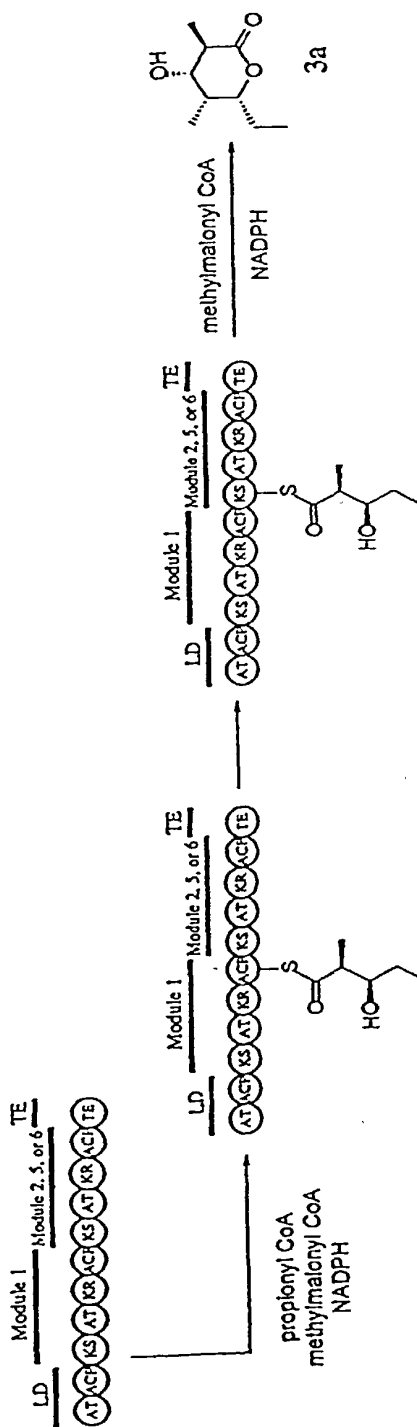
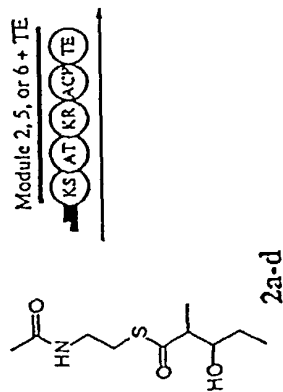
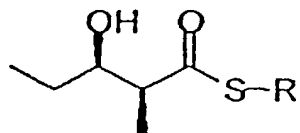
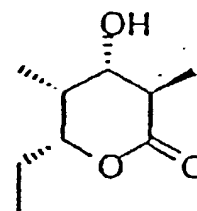


Figure 9

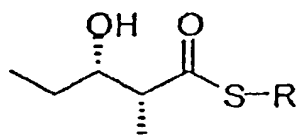




4a: R=Acyl carrier protein

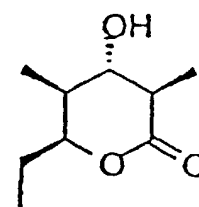


3a

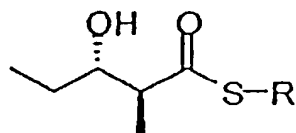


4b: R=Acyl carrier protein

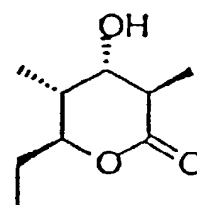
Module 6+TE



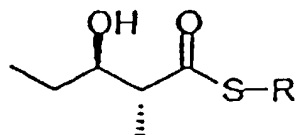
3b



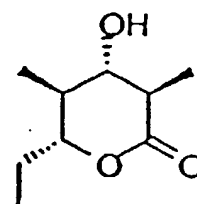
4c: R=Acyl carrier protein



3c



4d: R=Acyl carrier protein



3d

Figure 11

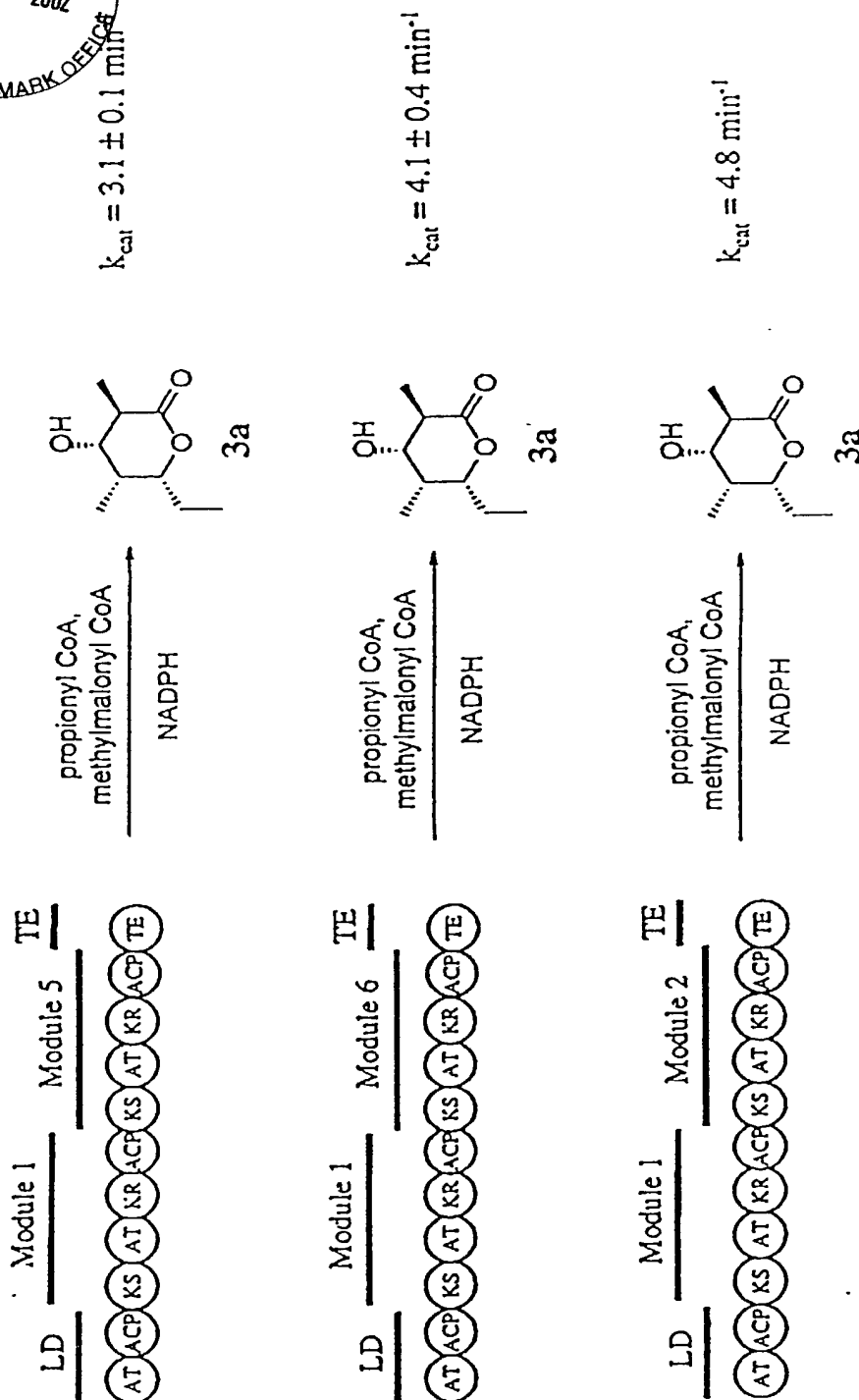


Figure 12






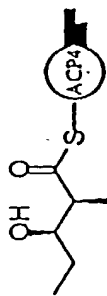
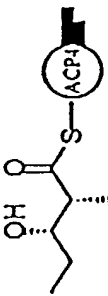
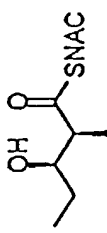
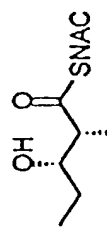
Substrate	(5)Module 2 + TE  KS AT KR ACP TE	(5)Module 5 + TE  KS AT KR ACP TE	(5)Module 6 + TE  KS AT KR ACP TE
4a 	2900 ± 500	290 ± 50	340 ± 60
4b 	18 ± 1	3.9 ± 0.7	85 ± 15
2a 	0.75 ± 0.01	0.016 ± 0.002	1.1 ± 0.1
2b 	0.0076 ± 0.0006	0.0011 ± 0.0001	0.058 ± 0.006

Figure 13



Substrate	(5)Module 2 + TE 	(5)Module 5 + TE 	(5)Module 6 + TE
4a 	6.7 ± 0.2	> 9.3 ± 1.4	> 10 ± 1
4b 	> 0.97 ± 0.02	> 0.48 ± 0.02	> 3.4 ± 0.4
4c 	> 1.0 ± 0.1	> 1.4 ± 0.1	> 2.1 ± 0.2
4d 	> 0.29 ± 0.03	> 0.20 ± 0.01	> 1.9 ± 0.1
2a 	> 4.6 ± 0.6	0.24 ± 0.01	17 ± 2.9
2b 	0.25 ± 0.02	0.017 ± 0.001	2.4 ± 0.2
2c 	N.D.	N.D.	N.D.
2d 	N.D.	N.D.	N.D.

Figure 14

1 2 3 4 5

Lane	ACP	Module	¹⁴ C-Label	Extender	Unit
1	holo-ACP4(4)	none	* 2 a	none	
2	none	(5)M2+TE	* 2 a	none	
3	none	(5)M2+TE	* 2 a	methyilmalonyl-CoA	
4	holo-ACP4(4)	(5)M2+TE	* 2 a	none	
5	holo-ACP4(4)	(5)M2+TE	* 2 a	methyilmalonyl-CoA	

← (S)M2-TE

← ACP4(4)

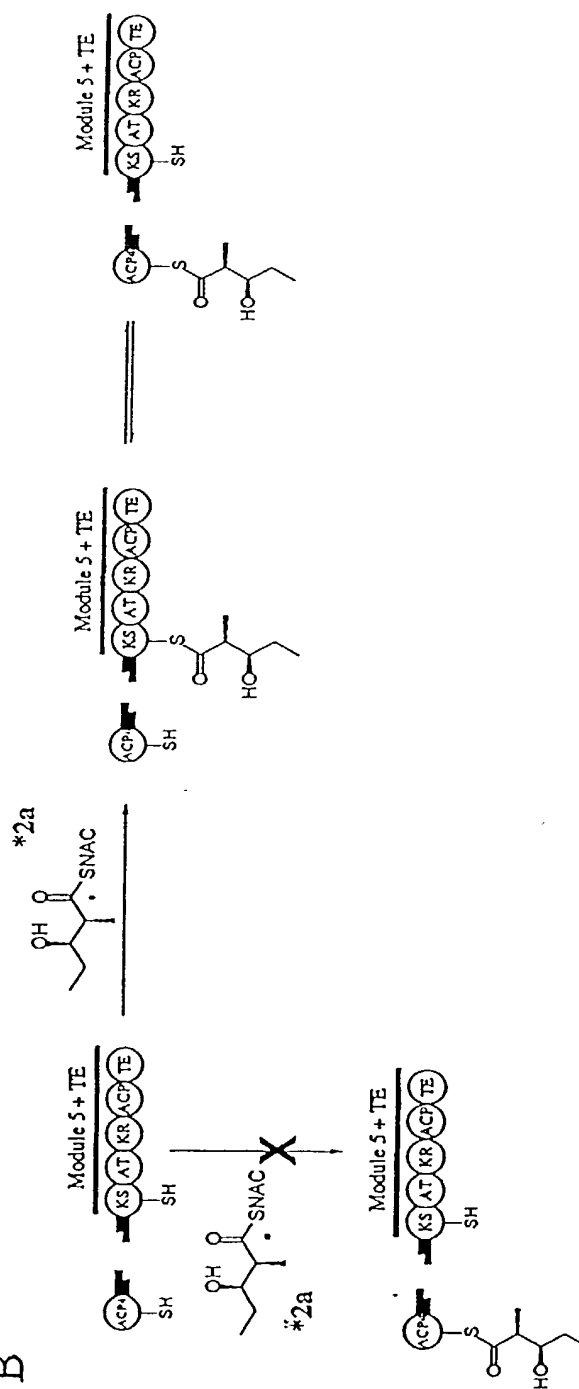


Figure 15

Figure 16

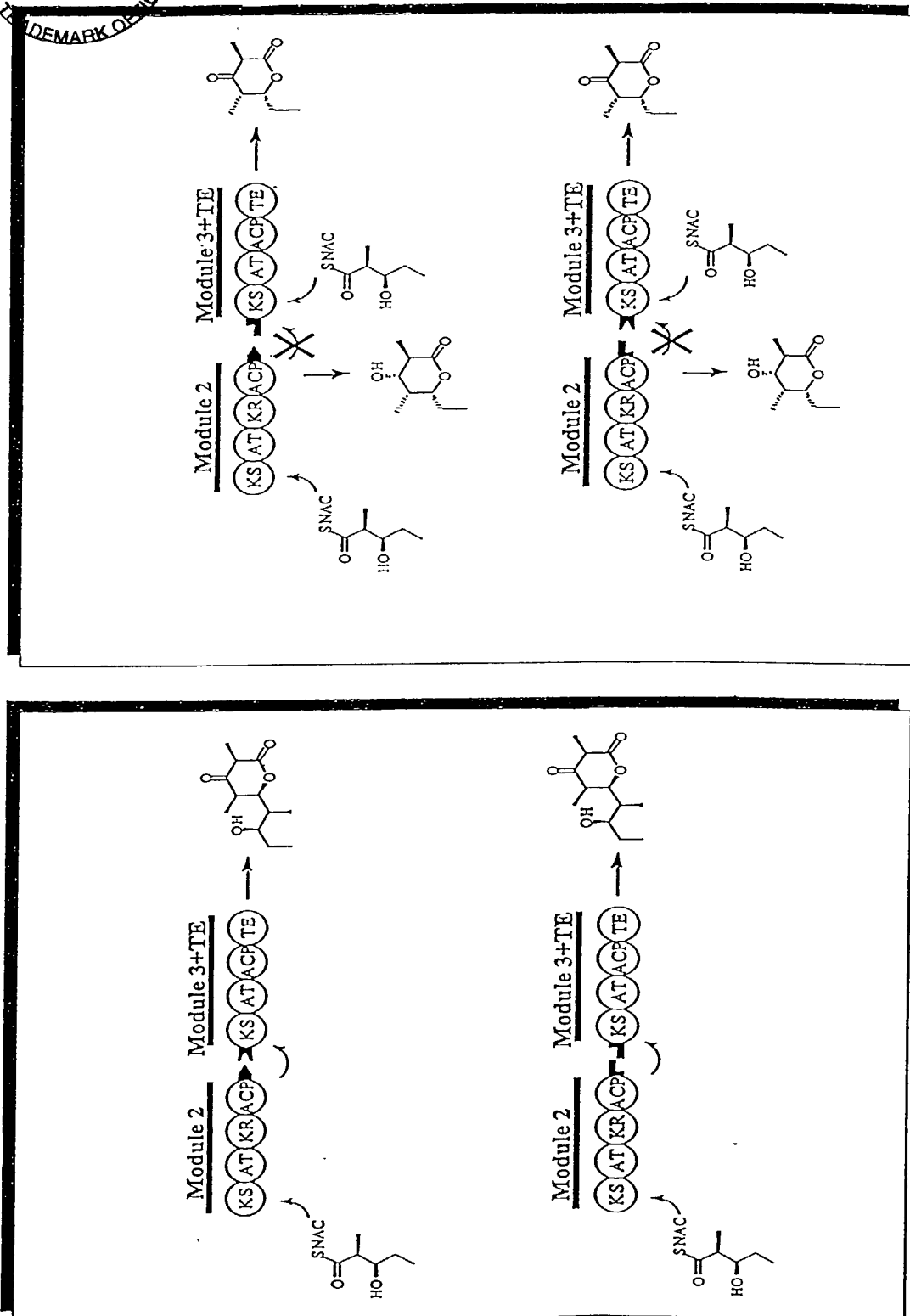
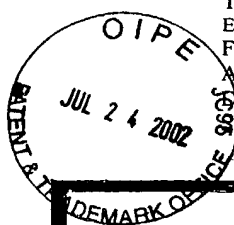


Figure 17

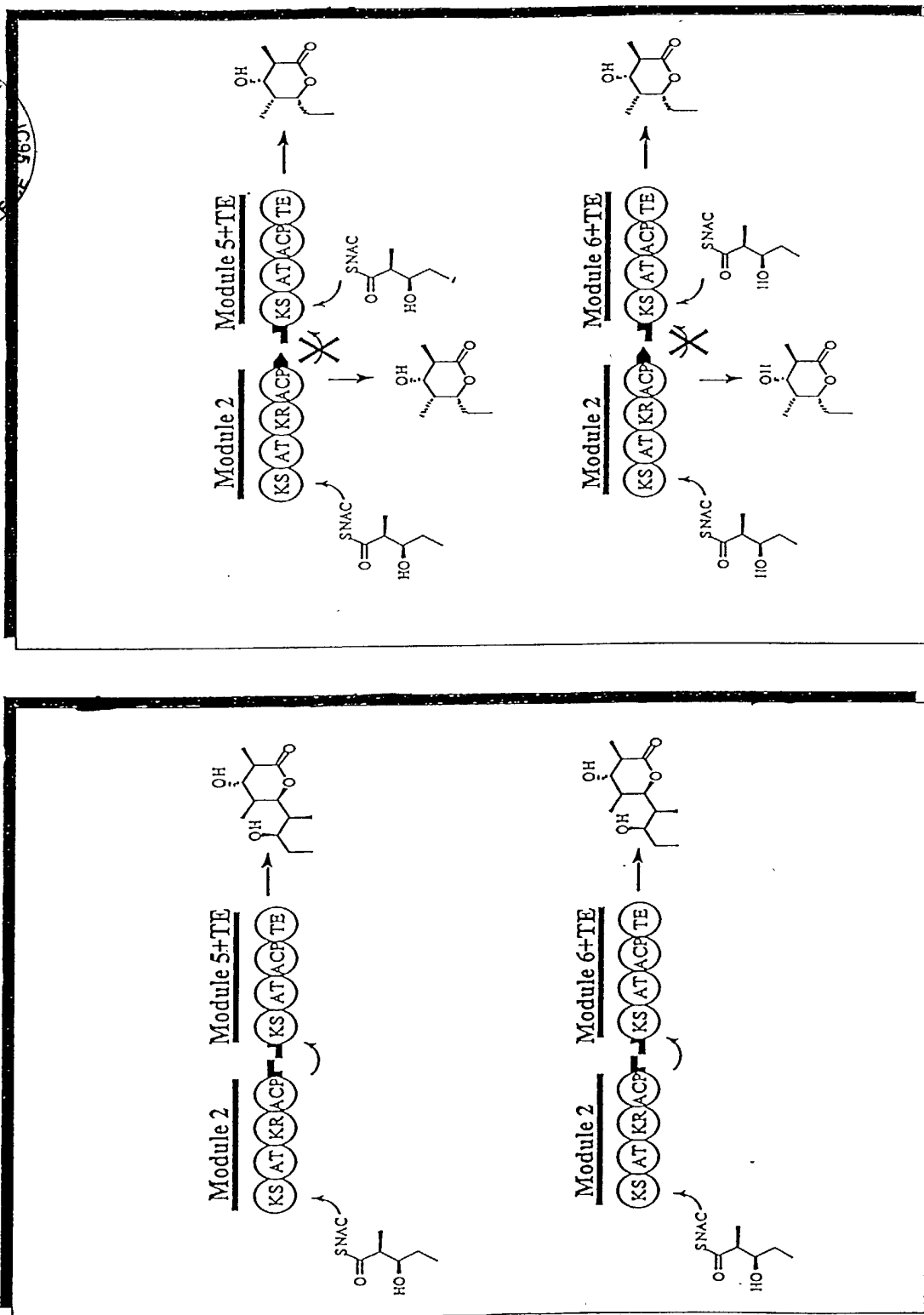
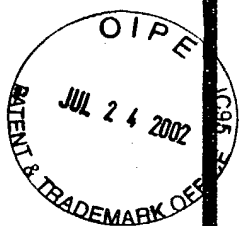


Figure 18

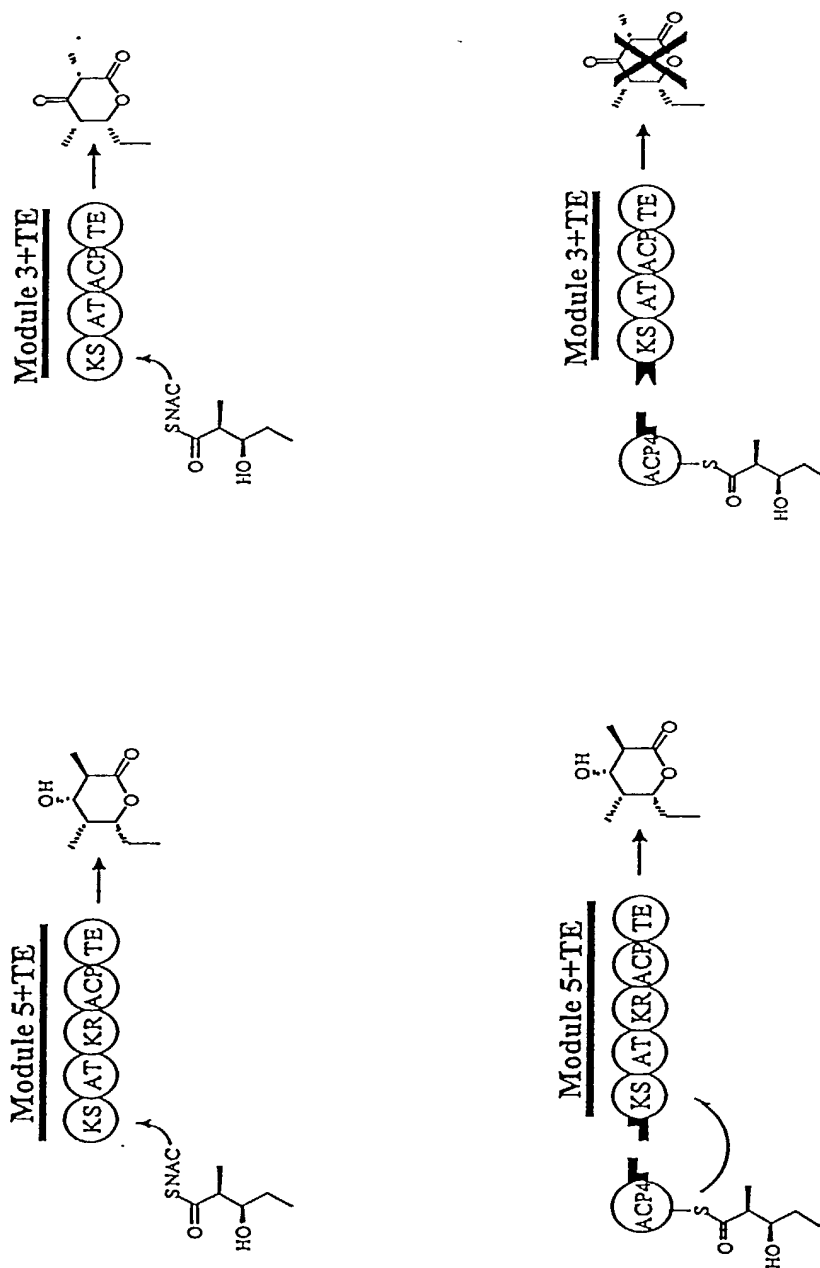


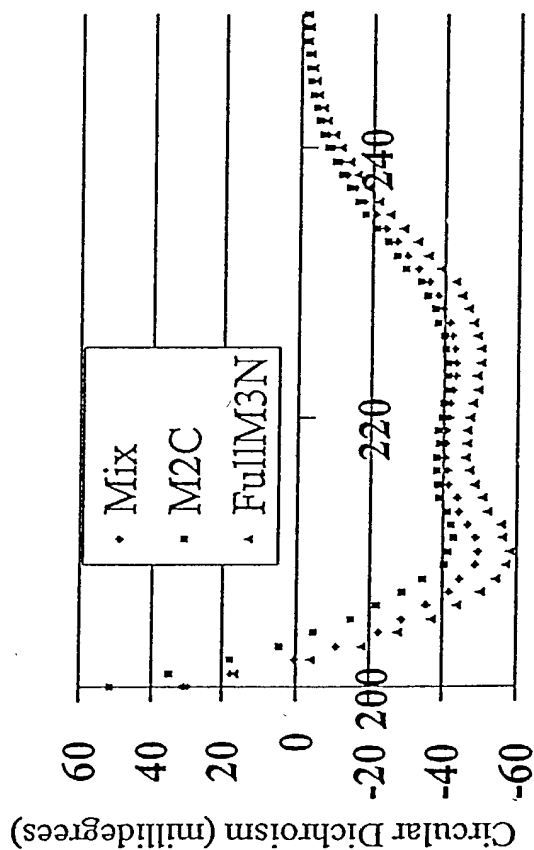
Figure 19



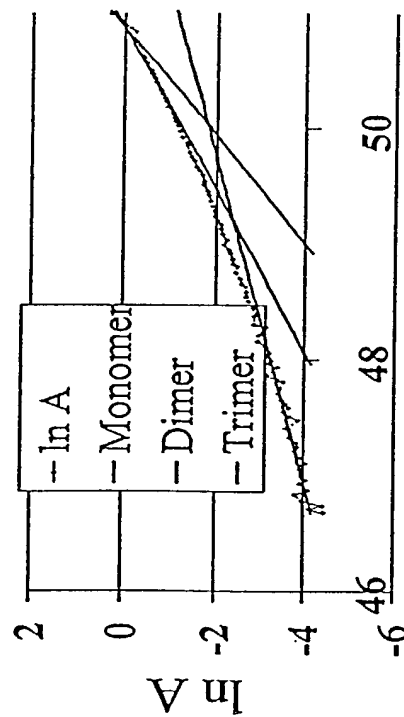
Substrates	Enzyme
 <chem>C[C@H]1C(=O)CC(C)(OC(C)(C)C)CC1</chem>	Module 2 + TE
 <chem>C[C@H]1C(=O)CC(C)(OC(C)(C)C)CC1</chem>	Module 5 + TE



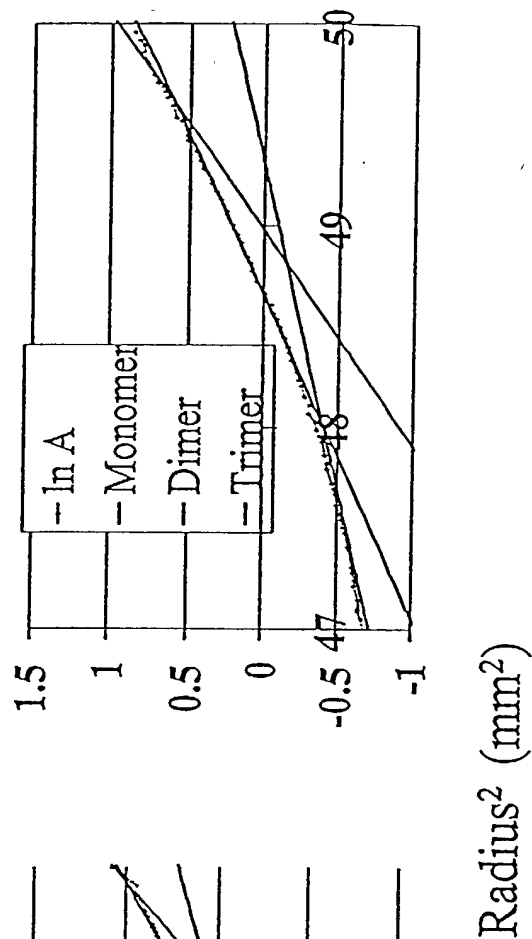
Figure 21



Module 2 C-terminus



Module 3 N-terminus



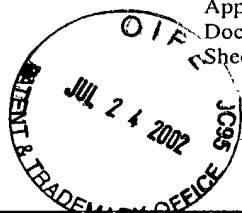


Figure 22

